Induction of cytotoxicity by chlorogenic acid in human oral tumor cell lines

Y. Jiang[1], K. Kusama[1], K. Satoh[3], F. Takayama[2], S. Watanabe[4] and H. Sakagami[2]

[1]Department of Oral Pathology, Meikai University School of Dentistry, Sakado, Saitama, Japan
[2]Department of Dental Pharmacology, Meikai University School of Dentistry, Sakado, Saitama, Japan
[3]Analysis Center, School of Pharmaceutical Sciences, Showa University, Hatanodai, Shinagawa-ku, Tokyo, Japan
[4]Seitoku Junior College of Nutrition, Katsushika-ku, Tokyo, Japan.

Summary

Millimolar concentrations of chlorogenic acid (CGA) showed higher cytotoxic activity against human oral squamous cell carcinoma (HSC-2) and salivary gland tumor (HSG) cell lines, as compared with that against human gingival fibroblast (HGF). The cytotoxic activity of CGA was significantly reduced by catalase or $CoCl_2$, but not affected by $FeCl_3$ or $CuCl_2$. ESR spectroscopy showed that higher (millimolar) concentrations of CGA produced radicals under alkaline conditions, acting as a prooxidant, whereas lower concentrations of CGA scavenged superoxide and hydroxyl radical. CGA produced large DNA fragments (as identified by slightly faster migrating band of DNA on agarose gel electrophoresis) and nuclear condensation (as demonstrated by Hoechst (No. 33258) staining) in tumor cell lines. Activation of caspase was demonstrated by staining with M30 monoclonal antibody, which reacts with degradation products of cytokeratin 18. Contact with CGA for at least 6 h was necessary for irreversible cytotoxicity induction. Pretreatment of the cells with caspase 3 inhibitor partially inhibited the cytotoxic action of CGA. These date suggest that CGA induces cytotoxicity in oral tumor cell lines, possibly by hydrogen peroxide-mediated oxidation mechanism.

Key words: Chlorogenic acid, oral tumor cells, radical, ESR, apoptosis, $CoCl_2$, catalase, caspase, DNA fragmentation

Introduction

Chlorogenic acid (CGA) (structure shown in Fig. 1) has been first isolated from coffee beans and distributed into fruits bodies and leaves of many dicotyledons and vegetables (Paganga et al., 1999). CGA has shown diverse biological activities, including anti-HIV activity (McDougall et al., 1998), antioxidant activity (Paganga et al., 1999, Yoshino and Murakami, 1998, Kono et al., 1998), anticarcinogenic activity (Morishita et al., 1997, Stich., 1992, Tanaka et al., 1990, 1993), modulating activity of cytochrome P450-linked enzyme (Teel and Huynh, 1998, Baer-Dubowska et al., 1998) and antiallergic activity (Ito et al., 1998). However, no detailed study of antitumor activity of CGA has been performed so far. We have recently shown that various polyphenolic compounds, such as epigallocatechin gallate (EGCG) (Ishino et al., 1999a), macrocyclic ellagitannins (Sakagami et al., 2000a), and isoprenoid-substituted flavonoids (Sakagami et al., 2000b) induced apoptotic cell death (characterized by DNA fragmentation and caspase activation detected with M30 monoclonal antibody (Caulin et al., 1997; Leers et al., 1999), in human oral tumor cell lines (human squamous cell carcinoma HSC-2, human salivary gland tumor HSG cells). We investigated here whether CGA shows the selective cytotoxicity against these tumor cell lines as compared with normal cells (human gingival fibroblasts) (HGF), and if so, whether CGA induces apoptosis-associated characteristics in the tumor cells.